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Abstract: **OBJECTIVES:** The aim of this study was to evaluate the effects of chemical activation of hydrogen peroxide (HP) gel on colour changes and penetration through the tooth structure. **METHODS:** One hundred and four bovine incisors were used. One dentine (CD) disc and one enamel-dentine (ED) disc were prepared from each tooth. They were positioned over artificial pulpal chambers and the bleaching was performed with an experimental 35% HP gel. Two control and six experimental groups were prepared. In the positive control group (PC) no chemical activator was used. In the negative control group (NC) the specimens did not receive any bleaching. Each experimental group received a different chemical activator (manganese gluconate-MG; manganese chlorite-MC; ferrous sulphate-FS; ferrous chlorite-FC; and mulberries root extract-MRE). After the bleaching procedure a sample of solution was collected from the artificial pulpal chamber and the HP concentration was measured. The data were analysed using ANOVA, Tukey's, and Dunnett's tests. **RESULTS:** The groups MG and FS showed a significantly lower penetration of HP than the PC group. For the parameter Delta E, all the groups, with the exception of the group MRE, showed a significantly higher means in relation to the PC group in ED colour. For dentine colour, just the groups MG and FS had significant differences in relation to PC. **CONCLUSIONS:** The addition of MG and FS decreases the penetration of HP. The chemical activation using metal salts tested was effective in increasing the bleaching effect.

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**Influence of chemical activation of a 35% hydrogen peroxide bleaching gel on its penetration and efficacy
“in vitro study”**

Influence of chemical activation on penetration and efficacy of dental bleaching “in vitro study”

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Keywords

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Influence of chemical activation of a 35% hydrogen peroxide bleaching gel on its penetration and efficacy “in vitro study”

Summary

Objectives: The aim of this study was to evaluate the effects of chemical activation of hydrogen peroxide (HP) gel on color changes and penetration through the tooth structure.

Methods: One hundred and four bovine incisors were used. One dentin (CD) disc and one enamel-dentin (ED) disc were prepared from each tooth. They were positioned over artificial pulpal chambers and the bleaching was performed with an experimental 35% HP gel. Two control and six experimental groups were prepared. In the positive control group (PC) no chemical activator was used. In the negative control group (NC) the specimens did not receive any bleaching. Each experimental group received a different chemical activator (Manganese gluconate - MG; Manganese chlorite - MC; Ferrous sulphate - FS; Ferrous chlorite - FC; and Mulberries root extract - MRE). After the bleaching procedure a sample of solution was collected from the artificial pulpal chamber and the HP concentration was measured. The data were analyzed using ANOVA, Tukey's, and Dunnett's tests.

Results: The groups MG and FS showed a significantly lower penetration of HP than the PC group. For the parameter Delta E, all the groups, with the exception of the group MRE, showed a significantly higher means in relation to the PC group in ED color. For dentin color, just the groups MG and FS had significant differences in relation to PC.

Conclusions: The addition of MG and FS decreases the penetration of HP. The chemical activation using metal salts tested was effective in increasing the bleaching effect.

1. Introduction

Dental bleaching is one of the most required esthetic treatments. It is based on the ability of hydrogen peroxide to penetrate the tooth structure and produce free radicals that oxidize the colored organic molecules.¹ The bleaching treatment can be performed using the at-home technique, which employs a low concentration hydrogen peroxide or carbamide peroxide as an oxidizing agent and is applied over a long period, or using the in-office technique, which requires a highly concentrated hydrogen peroxide bleaching gel, generally 35%, and is applied over a short periods.² It should be noted that a single application of in-office bleaching is usually not sufficient to achieve the desired bleaching results.³ Because of this it is necessary to apply the bleaching in multiple appointments for obtaining optimal results.² Therefore, it is very important for this technique to achieve more pronounced effects in a shorter period of time. This is the reason for using highly concentrated gels. In addition, the levels of free radicals in a hydrogen peroxide solution increases in a concentration-dependent manner.⁴

Taking into account these aspects, it is very important to study ways of increasing the efficiency of the procedure by stimulating the dissociation of hydrogen peroxide and the formation of free radicals. The most common way to dissociate hydrogen peroxide is to use a physical activation method, providing energy for the reaction. This can be done either through direct diffusion using an electric heating device or, more recently, using light sources, such as the blue light produced by quartz-tungsten-halogen lamps, plasma arc lamps, light emitting diodes, or lasers.² However, the potential increase in efficacy of bleaching gels by light have been questioned, and some studies have pointed that the benefit of its use is limited or of no significance.² Some studies showed that the uptake of H_2O_2 in the pulpal chamber was greatly enhanced by the application of heat.⁵ Moreover, the probability of cervical reabsorption was increased after a thermo-catalytic bleaching procedure associated with highly concentrated solutions was used in intracoronal bleaching.⁶

Furthermore, the heating of the bleaching gel also results in the heating of the pulpal tissue, increasing the occurrence of post-operative sensitivity and the risk of irreversible pulpal damage.² Another problem is that the heating of bleaching gel can increase the penetration of hydrogen peroxide through enamel and dentin⁵, increasing the potential of pulp irritation. Although this increase in penetration can improve the bleaching of dentin, which has a high influence in the color change with the dental bleaching procedure⁷, the diffusion of peroxide into the pulp also leads to an oxidative stress that can negatively affect the pulpal cell metabolism.²

The hydrogen peroxide is a very unstable molecule, and when in contact with tooth structure or organic molecules it undergoes dissociation.⁸ Another factor that can affect its dissociation is the pH in the peroxide solution or gel. Under alkaline pH conditions, radical formation from hydrogen peroxide is more likely and faster compared to acidic conditions. In addition, some enzymes and salts of transition metals, such as Fe, Cu, Cr, or Mn can act as a catalyst or “booster,” promoting the dissociation of the hydrogen

peroxide molecules and formation of free radicals.⁹⁻¹⁴ Some studies show that the association of metal salts with the bleaching gels were able to improve the dental bleaching effect.^{9, 11-13, 15} Other studies also suggest the use of enzymes, such as catalase, peroxidase, and dopamine, and the mulberry root extract as chemical activators.^{9, 16} The mulberry root extract contains phenyl flavonoid, which is effective as a human skin whitener. One of the biggest advantages of the chemical activation of hydrogen peroxide is the lack of pulpal heating during the process, thus reducing the harmful potential of the in-office bleaching procedure. In addition, it can have some influence on H₂O₂ penetration since a reaction is expected to happen between it and the chemical activator.

Due to the risks represented by light activation, the lack of studies showing its efficacy, and the possibility of using other more secured ways to stimulate the formation of free radicals, the aim of this study was to evaluate the effects different chemical methods to activate hydrogen peroxide had on color changes of tooth structure and the penetration of H₂O₂ through enamel and dentin. The null hypotheses analyzed were that the addition of chemical activator does not influence the color change and does not change the penetration of hydrogen peroxide.

2. Materials and Methods

2.1. Sample preparation

The specimens were prepared according to the method described by Wiegand et al.⁷ For that, one hundred and four extracted, non-damaged bovine intact incisors were stored in 0.1% thymol solution at room temperature until required. From each crown two enamel-dentin specimens with 3 mm in diameter were prepared from the labial surface with a trephine mill (Dentoflex, São Paulo, Brazil). In one of these specimens enamel was removed and the pulpal side was ground flat with water-cooled abrasive discs (1200 grit – FEPA-P; Struers, Ballerup, Denmark) in a polishing device (DP-10, Panambra, São Paulo, Brazil), resulting in dentin control specimens (CD) with a height of 1.2 mm. The thickness of the dentin disc was determined with a micrometer (Digimatic, Micrometer, Mitutoyo, Tokyo, Japan). In the second specimen, the labial and pulpal sides were ground flat until the remaining enamel and dentin layer of the enamel-dentin (ED) specimens reached 1.1 mm each (Figure 1). Preparing two specimens from each tooth allowed obtaining the dentin baseline color values. After preparation, the specimens were stored in 0.1 % thymol solution to avoid dehydration.

The specimens were embedded in composite resin. Therefore, the sample, except the top and the bottom surfaces, were acid etched for 15 s using a 35% phosphoric acid gel (3M/Espe, St. Paul, Mn, USA), and then washed for 30 s. The Single Bond 2 adhesive system (3M/Espe, St. Paul, Mn, USA) was applied according to the manufacturer's instructions and photocured for 10 s. The specimens were positioned in a silicon mold with a cavity 6 mm in diameter and 2 mm in depth. On the bottom of the mold there was a second level cavity 3 mm in diameter and 0.1 mm in depth (Figure 1). The ED specimens were positioned

inside the internal cavity with the enamel surface facing the bottom of the mold. The mold was filled with a flowable composite resin, Opallis Flow (FGM, Santa Catarina, RS, Brazil) color A1, and then photocured for 40 s. The specimen was removed from the mold, placed over a flat surface with the enamel side upward and photocured in for an additional 40 s. On the side of the mold there is a projection in the shape of a line which produces a lateral groove on the specimen. This helps to achieve the correct position for the moment of color reading (Figure 1). The specimens were attached to a metal holder and 0.1 mm of enamel was removed by polishing with 1200, 2400, and 4000 grit abrasive papers. The dentin side of the specimens was abraded with a 1200 grit abrasive paper, removing 0.1 mm of dentin and resulting in a specimen of 1 mm of enamel and 1 mm of dentin (Figure 1). The dentin side was acid etched for 15 s with a 35% phosphoric acid gel to remove the smear layer and open the dentinal tubules, simulating the real condition of the dentin in contact with pulpal tissue.¹⁷

A similar procedure of embedding was performed with the CD discs, using a silicon mold 6 mm in diameter and 1 mm in depth, with and a second level cavity 3 mm in diameter and 0.1 mm in depth. The upper part of the specimen was polished, removing 0.1 mm of dentin. The bottom part was abraded, removing 0.1 mm of dentin, to make a flat surface, resulting in a specimen 1 mm thick. However, the dentin side was not acid etched (Figure 1).

2.2. Initial color measurement

Prior to treatment, the baseline color of ED and CD specimens were assessed at standardized conditions according to the CIE-Lab system using a spectrophotometer CM2600-d (Konica Minolta, Osaka, Japan) with an integration sphere. The device was adjusted to use the D65 standard light source with 100% UV and specular reflection included (SCI). The observer angle was set at 2° and the device was adjusted to small reading area (SAV). The samples were carefully dried with an absorbent paper and immediately placed into individually prepared metal holders with a 2 mm diameter reading window, which allows for exact and repeatable positioning of each specimen and color measurement under standardized conditions.¹⁸ This holder has a lateral screw which connects to the lateral specimen groove allowing the repeatable positioning of the specimen. The color of each sample was measured three times and averaged. The results of color measurements were quantified in terms of three coordinate values (L^* , a^* , b^*) established by the Commission Internationale de l'Eclairage (CIE), which locates the color of an object in three dimensional color space. The L^* axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The a^* plane represents the degree of green/red color, while the b^* plane represents the degree of blue/yellow color within the sample. The mean L^* values of each ED specimen was used for stratified allocation of all samples among the various experimental groups. The L^* , a^* , b^* values of CD samples served as baseline control for the original color of dentin samples.

2.3. Bleaching experiment

For the bleaching procedure, the specimens were fixed in transparent Epoxi wells (Stycast, Emerson & Cuming, Pratteln, Switzerland) with two cavities of different diameters. The external cavity has 6 mm in diameter and 1 mm in height. The internal cavity has diameter of 3 mm and 2.83 mm in depth, with an internal capacity of 20 μ l to simulate a pulpal chamber (Figure 1). Before positioning of the DE disc, 20 μ l of 2 M acetate buffer (pH 4.5) was put inside the well to simulate the pulpal fluid and collect hydrogen peroxide that penetrated through the enamel and dentin. The acetate buffer was used to absorb and stabilize any peroxide that had penetrated until the moment of hydrogen peroxide measurement.¹⁹⁻²¹ After that, the specimens were positioned into the wells, with the dentin side in contact with the solution, and the margins were sealed with melted wax to prevent the bleaching gel to penetrate through the interface. The effectiveness of sealing the margins with wax was tested in a pilot study, where specimens made with composite resin without tooth structure were used. No penetration of hydrogen peroxide was detected inside the chambers.

The dental bleaching was performed according to each group's chemical activator, using an experimental 35% hydrogen peroxide bleaching gel. This concentration was chosen because it is the most frequently used in many commercial products for in office bleaching technique. This gel is composed of two parts. The first one is a solution of 50% hydrogen peroxide containing an acrylic thickener, which in an acidic environment is a white solution (solution A - pH 1.5). The second part consists of an aqueous solution containing an alkaline substance (solution B - pH 11.3) in which the chemical activator was added. For obtaining the final bleaching gel (pH 6.5) three parts of solution A and one part of solution B in volume were mixed in a mixing well. To have a guarantee of the right concentration of H₂O₂ on the pure 50% wt H₂O₂ solution (Sigma-Aldrich, Buchs, Switzerland) used to prepare the gel, the titration method using 0.1 N potassium permanganate solution was used. Primary standard sodium oxalate was used to standardize the 0.1 N KMnO₄.⁸ The teeth were allocated to eight groups (n = 13) according to the chemical activator added. To choose the concentration to be added, a previous test was performed to know the minimum of each chemical substance that could be added to produce a visible bubbling, and the maximum to keep the material in a gel state instead of foam. The bleaching was performed in a humid atmosphere. The metal salts used as chemical activators are not toxic in regular uses and right concentrations. They are used as nutritional supplements in human diet.

Two control and five experimental groups were prepared. In the positive control group (PC), the bleaching gel was applied over the surface of the ED specimens in a 2 mm thick layer (approximately 0.1 g). It remained on the surface for 10 min, being gently stirred after 5 min using a plastic instrument to dislodge the bubbles formed. The gel was removed using a vacuum aspirator and a new layer was applied following the same steps described. The second layer of gel was removed and a third layer was applied, in the same way as the previous two. This means that the bleaching gel remained on the enamel surface for a total of

30 min, simulating the most common protocol used for the commercial products. In the negative control group the specimens were positioned in the wells, with acetate buffer, in the same manner as the other groups, but did not receive any bleaching treatment. They were stored in artificial saliva during the same time used for the bleaching procedures in the other groups. Each experimental group received a different kind of chemical activator, which was added to 10 ml of solution B (Table 1). The application protocol was the same as the positive control group.

2.4. Peroxide measurement

After the first bleaching procedure of 30 min, the bleaching gel was removed with tap water and cotton wool balls. The specimens were stored in artificial saliva formulated for Gohring²² for 3 h and 30 min together with the wells, and then the same bleaching protocol was applied. They were again immersed in artificial saliva for 10 min. After this time the specimens were removed from the wells and stored in 2 ml of artificial saliva inside the Eppendorf tubes. After that, 5 μ l of the acetate buffer was collected using an automatic micropipette and transferred to acryl-cuvettes of 1000 μ l (Sarstedt, Nümbrecht, Germany). The method proposed by Bauminger²³ and modified by Hanning et al.²⁴⁻²⁶ was applied to measure the hydrogen peroxide concentration in the solution. This method is a spectrophotometric analysis based on the reaction of 4-aminoantipyrin and phenol with H_2O_2 catalyzed by peroxidase. The inorganic peroxide is oxidized by peroxidase, releasing oxygen which oxidizes achromatic chromogenic hydrogen donors, changing the color of the solution from transparent to pink. The amount of hydrogen peroxide in the solution was measured analyzing the absorbance in a spectrophotometer at $\lambda = 510$ nm.²⁴⁻²⁶ Aminophenazone (4-aminoantipyrin; 4 mmol/l), phenol (24 mmol/l), and peroxidase (0.4 U/ml) dissolved in 0.1 molar phosphate buffer at pH 7.0 was used as the enzyme reagent. The reagents were stored at 4°C. A calibration curve was prepared with a standard H_2O_2 solution with a known concentration to make a relation between the absorbance of light and the concentration of H_2O_2 on each sample. Inside the cuvettes with the samples, 1000 μ L enzyme reagent was added and mixed. After 3 min, the optical density of the resulting pink solution was measured and the concentration of H_2O_2 in mg/l was calculated. The data were analyzed using ANOVA and Dunnett's test.

2.5 Final color measurement

The ED specimens were stored in artificial saliva for 24 h and the same bleaching procedure was repeated two more times with an interval of 3 h and 30 min. In total the specimens were bleached for 2 hours. The specimens were then immersed in artificial saliva for 5 days in order to promote rehydration and color stabilization. The color measurements of treated ED samples were performed. Finally, enamel of the ED specimens was removed by grinding and polishing, and the color change of the subsurface dentin (D) samples was assessed. To compare tooth color before and after treatment in reference to baseline values, color changes (ΔE) and differences (ΔL , Δa , Δb) were calculated, with the following color definitions

of respective positive (+) and negative (-) values: ΔL =(+) white, (-) black; Δa =(+) red, (-) green; Δb =(+) yellow, (-) blue. For determination of ΔE the following formula was used: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$. The data were analyzed with ANOVA and Tukey's tests. For the comparisons with the PC and NC, the Dunnett's test was used. All the analyses were conducted using the software Statistica for Windows (StatSoft, Tulsa, USA) with a level of significance at 5%.

3. Results

The one-way ANOVA showed no significant difference in the H₂O₂ concentration in the simulated pulpal chamber between the experimental groups ($p = 0.1805$, $f = 1.62$, $df = 4$). The means of hydrogen peroxide penetration and the results of Dunnett's tests are given in Table 2.

In Table 3 are given the results of one-way ANOVA for the comparison of Delta L , Delta a , Delta b , and Delta E values among the groups for the color of enamel and dentin together (ED), and just dentins (D). In Tables 4, 5, and 6 are given the results of Tukey's and Dunnett's test for Delta L , Delta b and Delta E , respectively.

4. Discussion

Because of the interhuman differences in the perception of color, visual shade assessment of human teeth lacks standardization that may be improved by the use of a spectrophotometer.²⁷ In one study, spectrophotometric shade determination of natural teeth was more accurate and more reproducible compared with visual shade assessment (83.3% compared to 26.6%).²⁸ Another study showed that 47.9% of the visual shade selections were identical between two evaluators, while the spectrophotometric shade assessments were identical in 89.6% of the cases.²⁹ These studies demonstrate the subjectivity of visual shade assessment. In contrast, in most cases the spectrophotometer is able to select an identical shade match with respect to all color determinations, and revealed a good intra-agreement.²⁹ Therefore, the computer-aided evaluation has a high reliability along with well-defined differences between measurement periods of the active agents used. This is advantageous for clinical recall examinations and studies of tooth color evaluation.³⁰

As in other in vitro investigations evaluating color changes after bleaching treatment, bovine teeth were used for specimen preparation.^{7, 31, 32} The use of bovine teeth is justified, as they are easier to obtain and standardize, considering the large quantity of teeth required and because of ethical difficulties involved in obtaining healthy extracted human teeth.³³ Dentin discs obtained from bovine teeth were used in previous perfusion studies in comparison with human teeth and were considered adequate for this kind of analysis.¹⁷

Enamel samples were 1 mm thick, according to the average enamel thickness in human maxillary incisors.^{34, 35} As the result of the size of bovine teeth it was possible to prepare two specimens 2 mm in diameter, one beside the other, which guarantees a homogeneous distribution of the samples among the experimental groups with respect to baseline color values. As the negative control group, without the bleaching, had a significant lower color variation in relation to the bleached groups, we can consider that the acetate buffer inside the well and immersion in artificial saliva has no significant effect on color measurement. As expected, the most pronounced changes could be observed in L^* and b^* values, which reflected the increase of lightness and reduction in yellowness by bleaching treatment. With regard to a^* values, only minimal changes in the red-green direction occurred, showing that a minor influence on total color change.^{7, 18} In addition, no significant differences were observed between the experimental groups. Therefore the results of a^* values were not presented.

To get a bleaching effect it is essential that the hydrogen peroxide be able to penetrate through enamel and dentin, reaching the chromogenic molecules in the tooth structure. However, this substance is toxic to the pulpal tissue and the biggest is the penetration of hydrogen peroxide inside the pulpal chamber, most aggressive is the bleaching treatment. In Table 2 is shown that the addition of manganese gluconate and ferrous sulphate produced a statistically significant reduction of hydrogen peroxide penetration in relation to the PC. This can be due to the fact that the metal salts decomposed the hydrogen peroxide and formed the free radicals which react with the tooth structure. This decomposition can be seen when the gel is mixed and a great amount of oxygen bubbles were formed. Therefore less hydrogen peroxide molecules were able to reach the artificial pulpal chamber. Another possibility is that the free radicals that penetrated through the tooth structure were not detected by the reaction used to measure the hydrogen peroxide.

A study evaluating an extract of dental pulp has shown that hydrogen peroxide can dramatically inhibit pulpal enzyme activity in direct contact with cells.³⁶ It was reported that peroxide penetration into the pulp may result in different levels of tooth sensitivity or bleaching efficacy.²¹ The reduction of penetration can probably diminish the negative effects of the bleaching procedure on pulpal tissue. It is important to take into account that in vitro studies may be limited to simulate the clinical conditions. In the vital pulp, the pulpal fluid pressure is capable of reducing inward diffusion of chemicals.³⁷ In addition, there are sufficient mechanisms in the pulp that protect the tissue from radicals generated from the reaction of H_2O_2 , that the available levels of H_2O_2 would be significantly reduced.³⁸ Therefore, the amount of H_2O_2 reaching the vital pulp may be less than in vitro conditions.

The effect of metal salts is related to the interference of these chemical activators in the activation energy of the H_2O_2 dissociation reaction, acting as catalyzers, which will increase the speed of reactions and reduce the minimum quantity of kinetic energy the reagent molecules require for the reaction to start, providing another pathway of lower energy.³⁹ Formation of free radicals from hydrogen peroxide

associated with iron ions occur according to the Fenton's reaction or the Haber-Weiss's reaction.^{40, 41} Henry John Horstman Fenton discovered in 1894 that several metals have special oxygen transfer properties, which improve the use of hydrogen peroxide. Since this discovery, the iron catalyzed hydrogen peroxide has been called Fenton's reaction. After addition of iron to a hydrogen peroxide solution, both substances react to generate hydroxyl radicals according to the following equations: $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \bullet\text{OH} + \text{OH}^-$; $\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \bullet\text{OOH} + \text{H}^+$. In the first reaction ferrous iron (II) is oxidized by hydrogen peroxide to ferric iron (III), a hydroxyl radical and a hydroxyl anion. Iron (III) is then reduced back to iron (II), a peroxide radical and a proton by the same hydrogen peroxide. After that, the hydroxyl radicals are going to oxidize staining compounds. The Haber-Weiss's reaction generates hydroxyl radicals from superoxide and hydrogen peroxide as shown in the following equations: $\bullet\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \bullet\text{OH} + \text{HO}^- + \text{O}_2$. The reaction is very slow, but can also be catalyzed by iron.

The Fenton's reaction was initially used in the treatment of agricultural soil and industrial residues in an attempt to eliminate the toxic composites present. The addition of one of the mentioned iron ions causes the occurrence of an increase in the oxidative force of hydrogen peroxide, resulting in an increase in its degradation speed, causing free radicals to form more rapidly.^{42, 43} A study evaluating free radicals in hydrogen peroxide solution observed that the addition of a chelating agent, which removes metal ions from H_2O_2 solution, makes the amount of $\bullet\text{OH}$ generated decrease linearly.⁴ Another study reported that the generation of the free radical $\bullet\text{OH}$ was significantly increased by the presence of tooth discoloration caused by blood products in relation to non-discolored teeth, and that this can be related to the ferrous iron available to the catalysis of hydroxyl radical production.⁴⁴

There are differences, however, in the manner in which composites derived from manganese act in relation to the iron derivatives. This being so, when mixing manganese gluconate and chloride with hydrogen peroxide, these chemical activators acted in a simple manner, accelerating peroxide degradation, forming water and free radicals without dissociating themselves or participating in the reaction. This does not occur with iron, which ends up participating in the reaction, making electron exchanges.³⁹ In order to develop a new chemical activated formulation, an in vitro study evaluated the efficiency of a bleaching gel containing 35% hydrogen peroxide associated with manganese gluconate. The results obtained indicated that the presence of the chemical activator increased the efficacy of hydrogen peroxide to the order of around 1.5 to 8 times, which is in agreement with the results of the present study.¹² Another recent in vitro study reported that the manganese gluconate was effective in increase the bleaching effect.¹⁵

As can be seen in Tables 4, 5, and 6, the ferrous and manganese compounds were effective in improving the bleaching effect in relation to the positive control group. The differences between the results can also be related to the amount of salts added to the bleaching gel, its solubility, and reactivity. For example, the ferrous sulphate was added in the lowest amount. However, it was the more reactive substance in the bleaching, with intense bubble formation and reduction of H_2O_2 penetration. On the other

hand, the mulberry root extract did not show significant effects on bleaching and penetration. In addition, no bubble formation was detected with this substance. In relation to the mulberry root extract, some studies observed a significant improvement of the bleaching effect.^{14, 15} However, they used an experimental extract obtained through a different method than the industrialized product used in the present study. It is probable some important substances for the bleaching activation were lost during its preparation.

Taking into account the potential of metal salts use as chemical activators to increase the bleaching effects and reduce the H₂O₂ penetration, this method of accelerating the bleaching procedure can replace the conventional light activation, eliminating some of its negative effects. Complementary studies to compare bleaching using chemical activation with other means of activation should be conducted, both in regards to bleaching effectiveness and pulp irritation, so that a safer treatment with the same efficiency can be offered. Another interesting aspect is that the increase in effectiveness observed with the use of chemical activators could allow lower concentrations of hydrogen peroxide to be used, thus guaranteeing the same process effectiveness and minimizing the irritating potential of the oxidant agent. Therefore, more laboratorial and clinical studies must be done to confirm these findings.

5. Conclusions

- The addition of manganese gluconate and ferrous sulphate to the bleaching gel decreases the penetration of hydrogen peroxide.
- The chemical activation using metal salts tested was effective in increasing the bleaching effect of the hydrogen peroxide gel.
- The chemical activation using mulberry root extract was not effective.

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Table 1 – Chemical activators added to the experimental groups.

Experimental Groups	Chemical formulation (CAS)	Manufacturer	Amount inside the solution B	Concentration inside the gel
Manganese gluconate - MG	$\text{CH}_2\text{OH}(\text{CHOH})_4\text{COO})_2\text{Mn}\cdot 2\text{H}_2\text{O}$	Gluconal – Purac, Campos dos	0.01 g	0.025%

	CAS: 7544100	Goitacazes, RJ, Brazil		
Manganese chlorite - MC	MnCl ₂ ·4H ₂ O CAS: 13446-34-9	Vetec Química Fina Ltda, Rio de Janeiro, RJ, Brazil	0.005 g	0.0125%
Ferrous sulphate - FS	Fe ₂ SO ₄ ·7H ₂ O CAS – 77-82-63-0	LabSynth Ltda, Diadema, SP, Brazil	0.0016 g	0.004%
Ferrous chloride - FC	Fe ₂ Cl ₃ ·6H ₂ O CAS – 10025-77-1	Vetec Química Fina Ltda, Rio de Janeiro, RJ, Brazil	0.005g	0.0125%
Mulberries root extract from Morus Bombycis - MRE	CAS – 94167-05-2	Ikeda Corporation, Tokyo, Japan	1g	2.5%

Table 2 – Means of H₂O₂ concentration (mg/L) inside the simulated pulpal chamber and results of Dunnett's test for the comparison with the positive control group.

GROUPS	Mean	Standard Deviation	Dunnett (PC Mean = 4,79 ±1,74)
Manganese gluconate	2.17	1.24	0.000*
Ferrous sulphate	2.17	1.04	0.000*
Ferrous chlorite	3.43	1.91	0.118
Mulberries root extract	3.52	1.74	0.162
Manganese chlorite	3.76	1.64	0.329

* Significant differences (p<0.05).

Table 3 – Results of one-way ANOVA.

Paramater	Color	df	MS	F	p-level *
Delta L	ED	4	0.38	4.27	0.004*
	D	4	0.26	2.75	0.035*
Delta a	ED	4	0.01	1.00	0.413
	D	4	0.00	0.41	0.799
Delta b	ED	4	1.84	12.56	0.000*
	D	4	1.22	9.77	0.000*
Delta E	ED	4	2.11	15.78	0.000*
	D	4	1.27	9.80	0.000*

* Significant differences (p<0.05).

Table 4 – Results of Tukey's and Dunnett's tests for Delta L in ED and D disks.

Group s	ED disks					D disks				
	Mean	SD	Tukey's Homogeneous groups*	Dunnett PC (0,65 ±0.22)	Dunnett NC (0,00 ±0.15)	Mean	SD	Tukey's Homogeneous groups*	Dunnett PC (0,41 ±0.05)	Dunnett NC (0,00 ±0.16)
MRE	0.71	0.23	A	0.983	0.000**	0.49	0.26	A	0.955	0.000**
MC	0.94	0.24	AB	0.054	0.000**	0.80	0.41	A	0.006**	0.000**
MG	1.00	0.28	AB	0.015**	0.000**	0.80	0.31	A	0.006**	0.000**
FC	1.00	0.41	AB	0.012**	0.000**	0.81	0.23	A	0.004**	0.000**
FS	1.19	0.32	B	0.000**	0.000**	0.82	0.32	A	0.003**	0.000**

* The groups accompanied by the same letters do not present significant differences, ** Significant differences (p<0.05).

Table 5 – Results of Tukey's and Dunnett's tests for Delta b in ED and D disks.

Group s	ED disks					D disks				
	Mean	SD	Tukey's Homogeneous groups*	Dunnett PC (-1.22±0.34)	Dunnett NC (0.05±0.06)	Mean	SD	Tukey's Homogeneous groups*	Dunnett PC (-0.94±0.28)	Dunnett NC (0.03±0.06)

FS	-2.16	0.44	A	0.000**	0.000**	-1.61	0.43	A	0.000**	0.000**
MG	-1.97	0.32	AB	0.000**	0.000**	-1.47	0.22	AB	0.000**	0.000**
MC	-1.83	0.39	AB	0.000**	0.000**	-1.17	0.31	BC	0.313	0.000**
FC	-1.74	0.40	B	0.003**	0.000**	-1.04	0.29	C	0.907	0.000**
MRE	-1.16	0.36	C	0.993	0.000**	-0.86	0.46	C	0.969	0.000**

* The groups accompanied by the same letters do not present significant differences, ** Significant differences ($p < 0.05$).

Table 6 – Results of Tukey's and Dunnett's tests for Delta E in ED and D disks.

Groups	ED disks					D disks				
	Mean	SD	Tukey's Homogeneous groups*	Dunnett PC (1.45 ± 0.31)	Dunnett NC (0.17 ± 0.04)	Mean	SD	Tukey's Homogeneous groups*	Dunnett PC (1.06 ± 0.22)	Dunnett NC (0.09 ± 0.04)
MRE	1.42	0.36	A	0.999	0.000**	1.04	0.45	A	0.999	0.000**
FC	2.07	0.39	B	0.000**	0.000**	1.36	0.22	AB	0.097	0.000**
MC	2.11	0.33	BC	0.000**	0.000**	1.47	0.37	BC	0.014	0.000**
MG	2.26	0.31	BC	0.000**	0.000**	1.71	0.26	BC	0.000**	0.000**
FS	2.51	0.43	C	0.000**	0.000**	1.84	0.44	C	0.000**	0.000**

* The groups accompanied by the same letters do not present significant differences, ** Significant differences ($p < 0.05$).

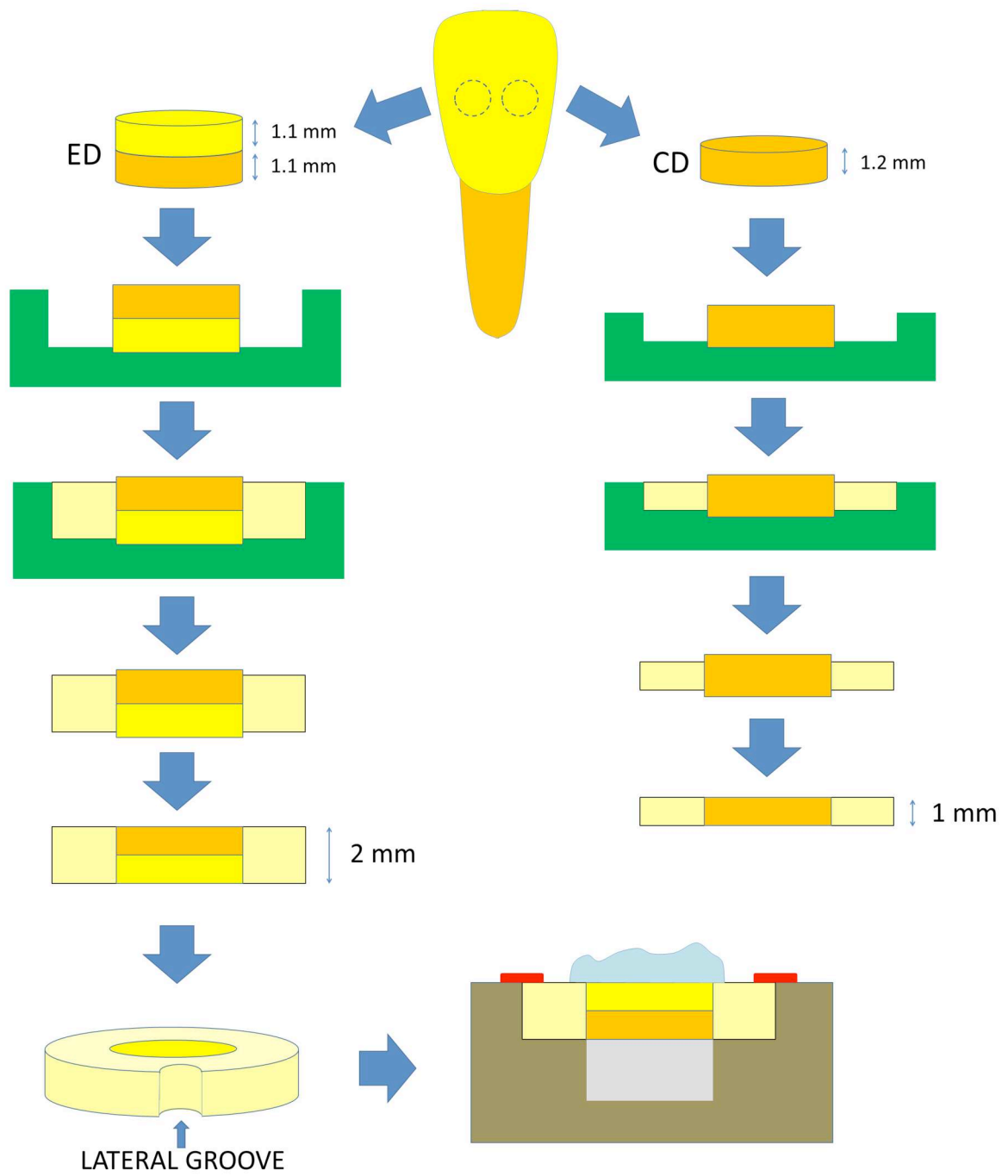


Figure 1 – Schematic drawing of the specimen preparation